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Decomposition studies in an easily-constructed microcosm: Effects of microarthropods and varying soil *p*H

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With 6 figures

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1. Introduction

Soil microarthropods are well suited for ecological studies since they occur in most terrestrial habitats, are easy to sample, include a high number of species showing a variety of adaptations and generally have short generation times.

A major limitation of field studies on microarthropods is, however, the great complexity of soil communities which include the macro- and microfauna, as well as a diverse microflora. More controlled laboratory studies are often necessary to single out the importance of one or a few environmental factors, but a large proportion of the laboratory studies have been performed under very artificial conditions (e.g. keeping the microarthropods on plaster of Paris, and feeding them with ordinary yeast). Anderson (1978) suggested that the interactions of species should be investigated within natural soil and litter habitats, containing the natural complement of microflora, in order to express the ecological niche of the animals rather than their ability to adapt to artificial conditions. Hence, undisturbed forest soil samples were defaunated and allowed to reestablish a natural microflora in the field before being maintained under controlled laboratory conditions to investigate interactions of mite species. Details are given here of a similar experimental system which is simpler to construct and use, and has proved to be very useful for keeping one or several species under controlled conditions for experimental studies. Results are presented from decomposition studies with and without microarthropods, using raw humus from coniferous forest. Effect of animals on soil *p*H was also followed, as well as the influence of *p*H on decomposition rate.

Preliminary information on the method was given in the poster session at the VIII. Int. Colloquium of Soil Zoology, Louvain-la-Neuve, 1982.

2. The microcosm

2.1. Construction

The microcosm consists of a cylindrical, open litter bag which is inserted into a lidded plastic container (fig. 1). The litter bag is 3 cm high, 3.4 cm in diam. and is made from a nylon cloth with 0.6 mm mesh size.[1]) The plastic container has an inner diameter of 3.5 cm and is 4 cm high.[2]) Five holes (of approx. 7 mm diam.) were drilled in the container; three in the wall, one in the bottom and one in the lid. Each hole was covered by a 20 mm × 20 mm piece of nylon cloth with 5 µm mesh size.[3]) When the microcosm is in operation, the lid can be completely sealed with waterproof, adhesive tape.

[1]) The nylon cloth is delivered by Swiss Silk Bolting Cloth Mfg. Co. Ltd., CH - 9425 Thal/SG, Switzerland. A great variety of mesh sizes is available, down to 5 µm. The litter bag is constructed by melting pieces of nylon cloth together with a soldering bit (fig. 2).

[2]) The boxes can be ordered from Dr. Franz Schneider & Co. KG, Kunststoffwerk, 8640 Kronach/Neuses, Germany (F.R.G.), („Filmrillendosen, Größe 2, glasklar").

[3]) "Tangit" glue was used. Only special glues adhere to plastic and nylon. Care must be taken that the glue is not absorbed into that part of the cloth which covers the hole.

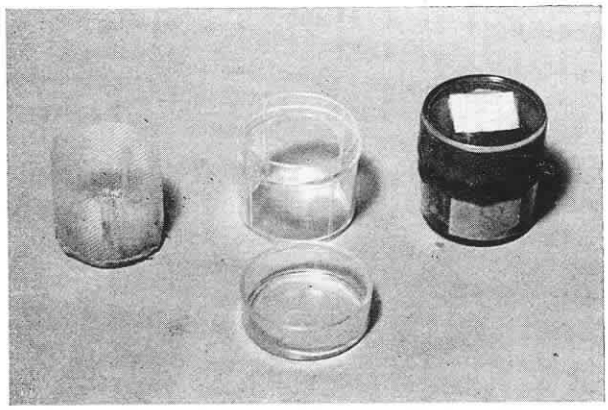

Fig. 1. The elements of the microcosm. From the left: Litter bag, lidded plastic container and the microcosm ready for use. Holes are covered with pieces of nylon cloth with 5 μm mesh size, and the lid has been sealed with waterproof, adhesive tape.

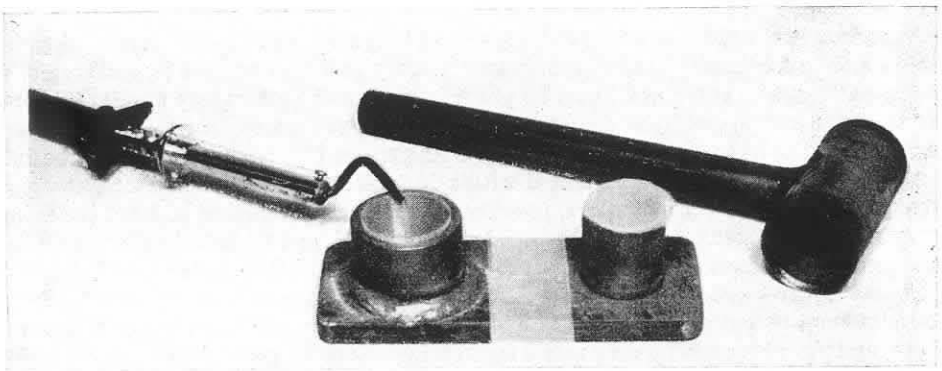

Fig. 2. Equipment for producing the cylindrical litter bags. A piece of nylon cloth is put inside a small metal cylinder, and the overlapping parts are fused with a soldering bit. The resulting nylon "ring" is placed around a compact metal cylinder (right), and a circular piece is fused onto one end. The hammer-like object is warmed in a gas flame and used to stamp out circular pieces of the nylon cloth.

The 5 μm cloth is an effective barrier for microarthropods. Inoculated animals stay in the culture, and no immigration is possible. Furthermore, gas exchange is sufficient, while water vapour escapes only very slowly. When kept in high air moisture, the culture does not need to be watered for many months.

2.2. Preparation, sterilization and inoculation of microflora

The procedure for handling experimental materials before the introduction of the animals is shown in fig. 3. Each litter bag was filled with about 2.3 g of mixed, dried raw humus from spruce [*Picea abies* (L.) KARSTEN] forest and inserted into the plastic container. The samples were then sterilized with gamma radiation (3.2 megarad). Before inoculation of the microflora, the raw humus was nearly saturated with distilled water.

The microflora was introduced in two steps (fig. 3). First, 0.2 ml of "soil extract" was added. This inoculum was produced by stirring a sample from the 0-layer in distilled water and afterwards filtrating the suspension through a cloth with 5 μm mesh size. In this way, a variety of bacteria was introduced together with a certain amount of fungal spores and small fragments of hyphae. This inoculation was done within a day after the moistening of the samples with distilled water and the "soil extract" was completely absorbed by the samples.

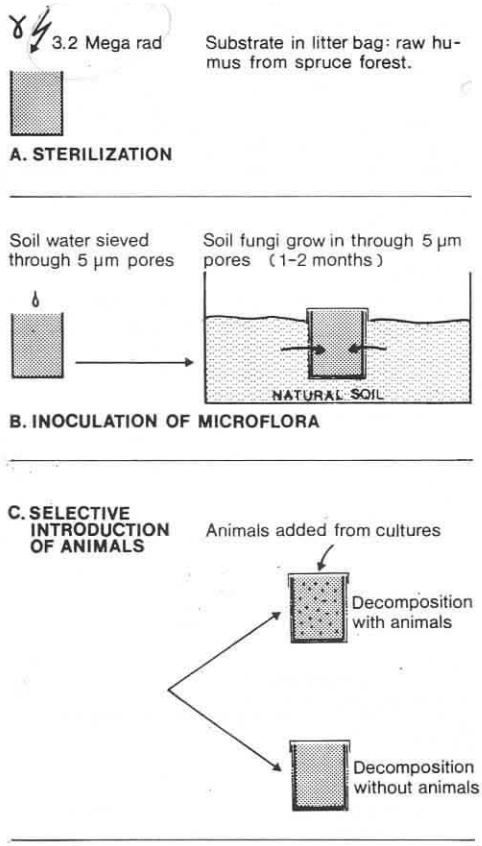

Fig. 3. Schematic illustration of the sterilization, inoculation of microflora and introduction of animals. Further explanation in the text.

Because Basidiomycete fungi may not be adequately inoculated by spore suspension, the units were placed in the field to allow hyphal invasion of the experimental material. After the lids had been sealed with tape, the containers were inserted into corresponding holes in natural forest soil (fig. 3). For practical reasons, this soil was kept indoors in plastic trays (at 15 °C). Soil fungi now colonized the samples through the fine-meshed holes in the wall and bottom. As most hyphae are 3—4 μm in diam., 5 μm mesh size is sufficient for colonization. Although the hyphae had to pass about 2 mm of air-filled space before reaching the contents of the inserted litter bag, the colonization evidently was intense. Dense "brushes" of hyphae were later observed protruding into the substrate from the holes. As several basidiomycetes are slow-growing, the samples were left in position for 1—2 months.

It is assumed that most nematodes and protozoa have free access to the experimental materials and some microfaunal groups may also have been added in the soil extract. Samples colonized by a natural microflora but completely free from soil animals cannot be achieved by this method.

2.3. Inoculation of microarthropods

When individuals of a certain species shall be added, great care must be taken that no small individuals of other microarthropod species are unintentionally introduced. For example, if fresh extractions from field soil are used, small Prostigmata may be introduced on the integument of larger animals. The most reliable procedure is to inoculate the experimental systems from pure cultures of the relevant species. These monocultures can be maintained in samples with the corresponding pretreatment as described above. Each "parent culture" can start with approximately twenty animals, which are carefully picked out from a field sample extracted onto a water surface.

When the microcosm is ready for inoculation of animals, the pure cultures are likewise extracted onto water. Most microarthropods survive for several hours on the water film, especially if the water is kept cool. Larger Cryptostigmata can be extracted onto a moist filter paper, as they tend to sink in water. Such large and slow-moving animals can be transferred to the microcosm with a needle or

Table 1. Information about four decomposition experiments with and without animals, at three different *p*H levels

Exper. No.	Fauna	Sampling I (after 3 months)			Sampling II (after 6 months)			Sampling III (after 12 months)		
		A	D	L	A	D	L	A	D	L
1	With *Schwiebea cf. nova* (Oudemans) (Acari, Astigmata)	14	14	14	15	14	15	14	14	14
	Control: Without animals							13	15	15
2	With *Mesaphorura yosii* Rusek (Collembola)	13	14	15	12	14	13	15	13	15
	Control: Without animals							15	15	15
3	With *Isotomiella minor* Schäffer (Collembola)	14	15	14	15	14	15	16	15	13
	With dry-extracted fauna (mainly microarthropods)								15	
	Control: Without animals							14	14	15
4	With dry-extracted fauna (mainly microarthropods)	24	22	24	24	21	24	26	26	26
	Control: Without animals							15	14	15

A = acidified soil, D = distilled-water treated soil, L = limed soil. The number of microcosms "harvested" at each sampling is given.

a small piece of filter paper. Smaller microarthropods on the water surface can be transferred in several ways. For slow-moving species, a small piece of filter paper held in tweezers is very useful. The filter paper is removed from the microcosm when the animals have moved into the substrate. Several larger species can be transferred by lifting them up on a curved needle, to which they cling when they feel contact. Very active and jumping Collembola cannot be transferred until the water is cooled down by a piece of ice below the vial. If possible, two stereomicroscopes should be used, to verify that the specimen is still present on the needle or bit of filter paper when arriving at the microcosm.

In experiments with several replications, care must be taken that the age distribution of introduced specimens is the same in all cultures. Because the viability of the animals can vary in different extractions from the "parent cultures", one should distribute animals from each extraction over all replications.

3. Experiments

3.1. Material and methods

Four decomposition studies were arranged, according to the plan in table 1. In each experiment, two factors were manipulated: the *p*H level of the raw humus, and the presence/absence of animals. Samplings were made after 3, 6 and 12 months. Microcosms without animals were harvested only at the last sampling.

Before the start, raw humus was adjusted to three *p*H levels (3.4, 4.4 and 5.2) by treatment with sulphuric acid, distilled water or $Ca(OH)_2$ as described by Hågvar & Abrahamsen (1980). Because this chemical pre-treatment might strongly affect the microflora, a complete sterilization by gamma-radiation was considered necessary. After sterilization and re-establishment of microflora (chapter 2.2.), animals were added from parent cultures: 30 per microcosm of the mite *Schwiebea cf. nova* and 20 per microcosm of each of the collemboles (*Mesaphorura yosii* and *Isotomiella minor*). Most animals were adults, but care was taken that the size distribution was similar in all microcosms. In experiments 3 and 4, a number of microcosms were started with a full microarthropod fauna. This was achieved by direct extraction into the microcosm from a raw humus core of similar size. Control of the extracted fauna revealed that also some enchytraeid individuals [*Cognettia sphagnetorum* (Vejdovsky)] were introduced by the dry extraction.

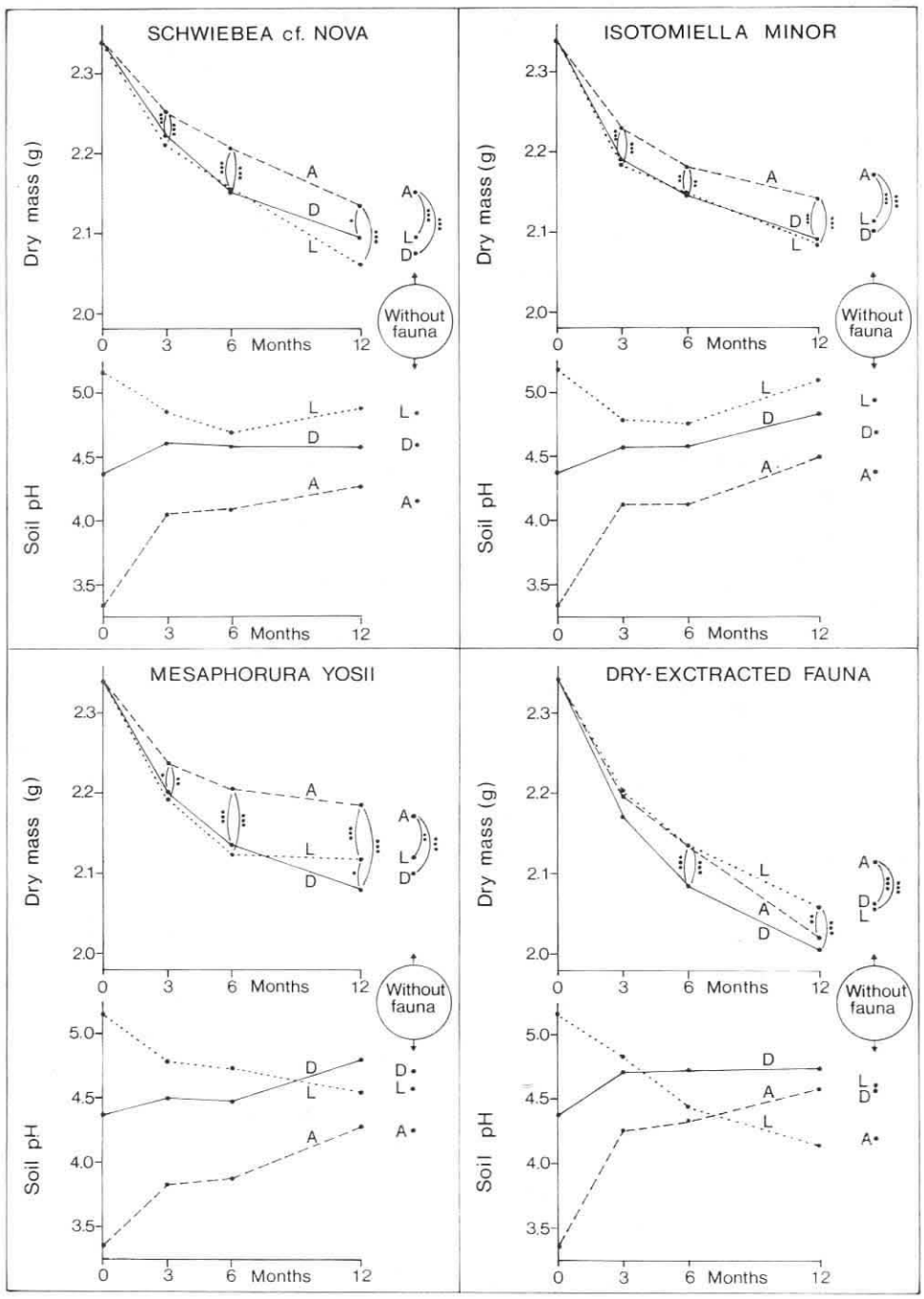

Fig. 4. Dry mass loss and soil pH changes in decomposition experiments with different fauna. Data from microcosms without fauna are given for comparison after 12 months. A = acidified soil, L = imed soil, and D = distilled — water treated soil. Significant differences are indicated: • = P $\leqq$ 0.05, •• = P $\leqq$ 0.01, ••• = P $\leqq$ 0.001.

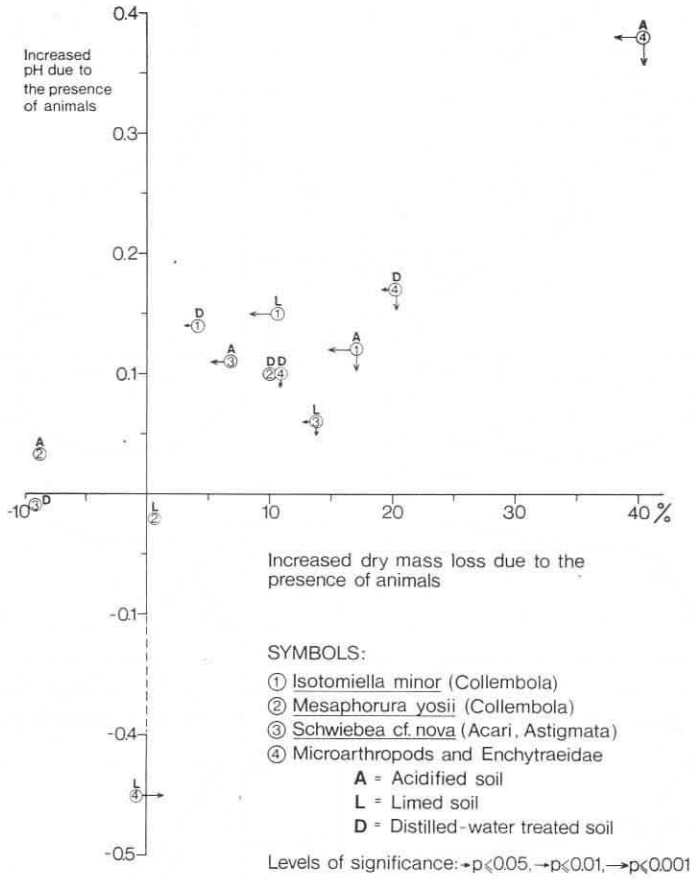

Fig. 5. Effect of animals on dry mass loss and soil pH in microcosm studies.

The microcosms were stored in the dark at 15 °C and approximately 80 % RH. Distilled water was added at intervals to keep the original moisture level.

After one year, all microcosms, including the controls or "animal free" treatment were extracted to establish population densities. Of 180 "control" microcosms, 81 % were still completely free from microarthropods, and only 3 % had so many animals that they were rejected (above 20 animals). In the monocultures of experiments 1—3, 4 % of the microcosms were rejected because some individuals of other species were present. The "full fauna" microcosms still contained a rich fauna after one year, but detailed numbers will not be given here. Populations in monocultures were high after one year, comprising more than one thousand animals per microcosm of *Schwiebea cf. nova* and *Mesaphorura yosii*, and more than two hundred animals of *Isotomiella minor*.

Table 1 shows the number of microcosms which were acceptable at each sampling and treatment.

3.2. Effect of soil pH on decomposition rate

Fig. 4 shows the changes in dry mass and soil pH during one year in all four experiments. Values are also given from the control cultures without animals at the last sampling. In microcosms with *Schwiebea cf. nova* and *Isotomiella minor*, soil pH in acidified, distilled water-treated and limed soil were different during the whole period. In these two experiments, dry mass loss was significantly slowest in the acidified raw humus at all three samplings. Also in the cultures without animals, the mass loss after one year was significantly lowest in acidified soil.

Cultures of *Mesaphorura yosii* similarly showed that acidified raw humus, both with and without animals, decomposed slowest at low pH levels. The same can be seen from control

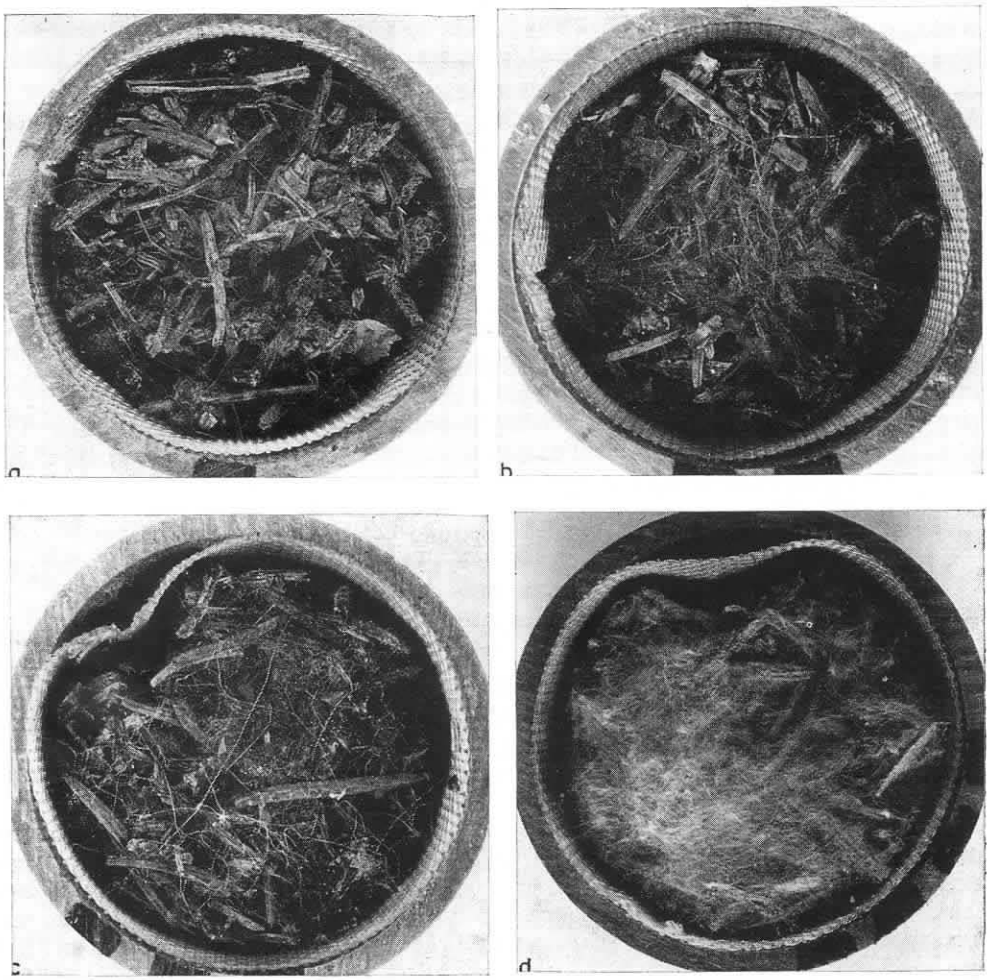

Fig. 6. Cover of fungal hyphae on the raw humus surface, divided into four classes.

cultures in the experiment with dry-extracted fauna. However, in these two experiments, limed samples showed a remarkable drop in pH towards the end of the year, becoming even lower than the acidified soil in "full fauna" samples. A simple relationship between pH and dry mass loss still existed at the end of these experiments, as remaining mass was highest in soil with lowest pH.

3.3. Effect of microarthropods on decomposition rate and soil pH

The data from the last sampling in fig. 4 show that microcosms with animals tended to have a lower dry mass and a higher pH than microcosms without animals. These relationships are shown more precisely in fig. 5. Nine of the thirteen plots were situated in the square showing both increased decomposition rate and increased pH in the presence of animals. Several effects were significant. Clearly, even single species could induce these changes. Effects of animals were independent of the original pH of the raw humus.

Fig. 5 also shows that in some situations, these effects were not achieved. Most atypic is the limed raw humus with microarthropods and enchytraeids, where soil pH was strongly reduced and decomposition rate unaffected (lowest plot).

Table 2. The amount of fungal hyphae on the surface of the raw humus samples after one year with an without dry extracted fauna

		A	D	L
Without animals	($\bar{x}$ ± SD)	1.60 ± 0.83	1.80 ± 0.94	1.73 ± 0.59
With animals	($\bar{x}$ ± SD)	0.19 ± 0.40	0.50 ± 0.71	0.38 ± 0.57

The mean cover of hyphae was quantified according to the rough scale 0—4 shown in fig. 6.
A = acidified samples, D = distilled-water treated samples, L = limed samples. In all three categories, significantly more hyphae occurred on raw humus without animals, compared to samples with animals ($P < 0.001$).

3.4. Effect of dry-extracted fauna on the cover of fungal hyphae

At the last sampling with dry-extracted fauna (experiment 4, table 1), the amount of fungal hyphae on the surface of the raw humus was roughly estimated. This was done in all microcosms, both with and without animals, according to a simple scale 1—4 as shown in fig. 6. In many cases, no hyphae could be seen on samples with fauna (value zero). At all *p*H levels, microcosms with animals had significantly less cover of hyphae than microcosms without animals (table 2).

4. Discussion

4.1. The method

The referred experiments confirm that the microcosm method works as intended for manipulations of fauna, substrate and climate. When using a slowly decomposing substrate as raw humus from coniferous forest, experiments may be run for several years and long-term studies can be performed on decomposition, on the effects of contaminated substrates on communities and species, or on processes like competition or predation. At any time, the litter bag may be taken out for measurements of O_2 consumption or CO_2 production. It is also possible to collect leachates below the microcosm for plant nutrient analysis.

Nygard & Solberg (1985) used this microcosm method to study competition between four collembolan species [*Isotomiella minor* in combination with either *Folsomia quadrioculata* (Tullberg) *s. l.*, *Mesaphorura yosii*, or *Onychiurus absoloni* (Börner)]. In all combinations, the population growth of both species was reduced compared to the population growth in monocultures. For their purpose they modified the method as follows: they used no litter bags and put undisturbed soil cores directly into the plastic containers similar to Anderson (1978). This gives natural soil conditions and a better contact with invading fungi, but larger variations in soil structure between replications. As they were not dependent upon full elimination of microflora before starting, "sterilization" was made by freezing to —80 °C for 24 h. Moisture was kept high by surrounding the glasses (except the top lid) with moistened vermiculite.

Compared to Anderson's (1978) microcosms, the present design has smaller units and finer mesh size. This allows for large numbers of replicates for faunal monocultures, excluding most other soil invertebrates.

In order to achieve a more homogenous microflora in the different microcosms, one might mix the substrate after the ingrowth of fungi and redistribute it into the experimental units. This treatment will not, however, give an exact dry mass at the start.

4.2. Effect of soil *p*H on decomposition rate

Field and laboratory experiments with artificial acid rain have shown that decomposition rates are often reduced in acidified soil (Bååth *et al.* 1980, Hågvar & Abrahamsen 1980, Hågvar & Kjøndal 1981). Also other studies, summarized by Hågvar (1986), are in accordance with the present results. While field experiments of this type are both demanding and expensive, valuable information can be achieved through simple microcosm arrangements.

4.3. Effect of microarthropods on decomposition rate and soil *p*H

The use of microcosms has several advantages compared to the two other methods commonly used for quantifying the role of microarthropods in decomposition processes. Much used is artificial confinement of substrate in litter bags of different mesh sizes. In this way, various animal groups can be excluded. However, important factors such as moisture, microbial development and the loss of fine fragments are easily affected by the mesh size (e.g. WITKAMP & OLSON, 1963; CURRY, 1969; ANDERSON, 1973; ST. JOHN, 1980). The other method, suppressing animal populations by chemicals, may also affect the microflora (e.g. WITKAMP & CROSSLEY, 1966; PARKER *et al.*, 1984). Furthermore, by introducing the animals manually, one has a much larger control over the fauna than by merely screening different size classes through various mesh sizes.

Most such experiments with litter bags or chemicals have given reduced decomposition rates in the absence of microarthropods (see review by SEASTEDT, 1984). The present results are of the same character, with several significant effects, but also cases where the animals did not increase decomposition rate. In one case, also the soil *p*H dropped markedly in the presence of dry-extracted fauna. These observations may either reflect that decomposition systems are rather unstable and the effect of animals may be unpredictable, or that the results may be due to atypical development in some of the closed microcosm systems.

The natural abundance of microarthropods in the relevant raw humus would correspond to approximately 300 animals per microcosm. The peak abundance of *Schwiebea cf. nova* and *Mesaphorura yosii* was more than three times this value, which might perhaps result in overgrazing of microflora. With only one species present, the system also lacks interactions with other soil animals. In any case, the experiments have shown that the presence of one small microarthropod species may increase the dry mass loss of coniferous raw humus, and a simultaneous rise of *p*H tends to occur in these cases. The reason for the *p*H increase is unclear. It is also questionable whether it is the direct effect of animals through grazing, etc. which stimulates decomposition, or whether increased decomposition rates are more due to the raised *p*H and improved conditions for the microflora. In microcosms without animals, the most acid raw humus always decomposed most slowly (fig. 4). This indicates that the *p*H rise induced by the microarthropods may have an important stimulating effect on the microflora.

4.4. Effect of dry-extracted fauna on the cover of fungal hyphae

Clearly, the presence of microarthropods and enchytraeids reduces the amount of visible hyphae on the raw humus surface. This may be due to grazing, but it may also be simply due to the surface activity of larger species. If they graze and disrupt the surface mycelium, this may become much less visible. In any case, the animals' activity makes it more difficult for the surface fungi to establish bridges to new microsubstrates. This effect may, however, be counteracted by continuous spreading of fungal spores via the body surface and excrements of animals. In addition, a reduced amount of aerial hyphae in the raw humus makes it easier for microarthropods to move freely around. Observations in connection with the present experiments have shown that the mobility of smaller microarthropods can be strongly reduced among a dense "web" of fungal hyphae.

5. Résumé

(Étude de la décomposition dans un microcosme facile à construire: Effets des microarthropodes et des variations du *p*H du sol.)

Un microcosme simple est décrit dans lequel microarthropodes et enchytraeides peuvent être élevés dans des conditions presque naturelles.

Le microcosme se compose d'un sac de litière cylindrique (3 cm de hauteur et 3,4 cm de diamètre) inséré dans un réservoir revêtu de plastique. Des trous ménagés dans le réservoir, couverts d'une tissu de nylon de 5 μm de maille, permettent un échange suffisant de gaz, tandis que l'au ne s'évapor que lentement. En utilisant dans le sac de litière un substrat à décomposition lente comme de l'humus

brut, le microcosme peut se maintenir durant des annés et ne demande que l'apport d'eau distillée à de rares intervalles. Après une stérilisation (par exemple par irradiation gamma) la microflore est introduite en partie par de l'eau édaphique filtrée à travers un tissu de nylon (5 μm de maille) et en partie en introduisant le microcosme dans le sol pour permettre aux hyphes de champignons de le coloniser au travers des mailles couvrant les trous du réservoir en plastique. Des microarthropodes sont ajoutés manuellement. Le microcosme permet des manipulations de substrat, de climat, de microflore et de mésofaune. Des résultats concernant le sol brut de forêt de sapin sont donnés. La vitesse de décomposition a été réduite dans des échantillons à pH bas dans la même mesure qu'en absence de microarthropodes.

La présence de microarthropodes (même d'une seule espèce), peut augmenter la vitesse de décomposition et, dans ce cas, on observe une augmentation du pH du sol. Dans les microcosmes avec microarthropodes et enchytraeides, la couverture d'hyphes à la surface du sol brut est fortement réduite.

Mots-clés: Microcosme, microarthropodes, mésofaune, décomposition, pH, hyphes.

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Synopsis: *Original scientific paper*
HÅGVAR, S., 1987. Decomposition studies in an easily-constructed microcosm: Effects of microarthropods and varying soil *p*H. Pedobiologia **31**, 293—303.

A simple microcosm is described in which microarthropods and enchytraeids can be kept under nearly natural conditions. The microcosm consists of a cylindrical litter bag (3 cm high and 3.4 cm in diam.) inserted into a lidded plastic container. Holes in the container, covered by a 5 μm mesh nylon cloth, allow for sufficient gas exchange, while water vapour escapes only gradually. Using a slowly decomposing substrate such as raw humus in the litter bag, the microcosm can be run for several years and needs only distilled water added at rare intervals. After sterilization (for instance by gamma radiation), the microflora is introduced partly via a soil/water suspension sieved through a 5 μm mesh nylon cloth, and partly by inserting the microcosm into the soil to allow fungal hyphae to grow in through the fine-meshed cloth covering the holes in the plastic container. Microarthropods are added manually. The microcosm allows for manipulations of substrate, climate, microflora and mesofauna. Results are given with raw humus from spruce forest as substrate. Decomposition rate was reduced in low *p*H-samples, both in the presence and absence of microarthropods. The presence of microarthropods, even one species, could increase the decomposition rate, and in these cases soil *p*H also increased. In microcosms with microarthropods and enchytraeids, the cover of hyphae on the raw humus surface was strongly reduced.

Key words: Microcosm, microarthropods, mesofauna, decomposition, *p*H, hyphae.